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TXR#: 0051360

DATA EVALUATION RECORD

STUDY TYPE: developmental toxicity rats
OPPTS Number: 870.3700 OPP Guideline Number: §83-3 (b)
DP BARCODE: D SUBMISSION CODE: S
P.C. CODE: 030066
TEST MATERIAL (PURITY): 2,4-D isopropyl ester (97.12% a.i.)
CHEMICAL: isopropyl (2,4-dichlorophenoxy) acetate; 2,4-dichlorophenoxyacetic acid, isopropyl ester
SYNONYM: 2,4-D IPE
CITATION(s): Nemec, M. D., (1994) A Developmental Toxicity of 2,4-D-Isopropyl Ester in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233004, January 11, 1995. MRID 43523101. Unpublished

Nemec, M. D., (1994) A Dose Range-Finding Developmental Toxicity of 2,4-D-Isopropyl Ester in Rats. WIL Research Laboratories, Inc., Ashland, OH. Laboratory Study No. WIL-233003, October 26, 1994. MRID 43523001. Unpublished

SPONSOR: California Citrus Quality Control/John Wise & Associates, Ltd. [representative]

EXECUTIVE SUMMARY: In a developmental toxicity study [MRID 43523101 +43523001], pregnant female Sprague-Dawley Crl:CD®BR rats [25/group] were administered the isopropyl ester of 2,4-dichlorophenoxyacetic acid [99.4%] *via* gavage at dose levels of 0 [100% corn oil], 10 mg/kg/day, 30 mg/kg/day, and 100 mg/kg/day [acid equivalents] from gestation day 6 through gestation day 15.

There were no abortions or premature deliveries. There was one death; a high-dose dam died on gestation day [GD] 17. Clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis] were observed in this dam one to five days prior to death and in two other high-dose dams between GD 11 and GD 14. There was a slight decrease in body weight [93%-96% of control] from GD 7 through GD 20 at the high-dose level, and terminal body weights were 95% of control at this dose. There was a dose-related decrease in body-weight gain during the dosing period, with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. Corrected body-weight gains were significantly lower at the high-dose level [83% of control] compared to the controls. During the dosing period, there was a decrease in food consumption at the mid- [91% of

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control] and high- [77% of control] dose levels.

Pregnancy rate was comparable among the groups, although the high-dose dams [88%] displayed the lowest rate [control (100%), low (96%), and mid (100%)]. The numbers of corpora lutea/dam, implantation/dam, and live fetuses/dam were comparable among the groups. There were no dead fetuses. One high-dose dam totally resorbed a litter of 11[10 early resorptions; 1 late resorption]. Fetal body weights at the high-dose level were lower than the control [89% of control]. There was a significant decrease in the percent of female fetuses [40.8%] at the high-dose level compared to the concurrent control [49%] and historical control [43.3%-58.4%]. There was no increased incidence of external or visceral malformations or variations. There was a significant increase in the incidence of skeletal malformations [7th cervical ribs and 14th rudimentary ribs] at the high-dose level compared to the control.

The maternal toxicity NOAEL is 10 mg/kg/day, and the maternal toxicity LOAEL is 30 mg/kg/day, based on decreased body-weight gain and food consumption during the dosing period. At the highest dose tested [100 mg/kg/day], one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis], and one litter with 100% resorptions were observed.

The NOAEL for developmental toxicity is 30 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on a decreased fetal body weight, an increased incidence of skeletal malformations [7th cervical ribs and 14th rudimentary ribs], and a decreased percent of female fetuses.

This guideline developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline requirement [OPPTS 870.3700; §83-3(a)] for a developmental toxicity study in the rodent.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging, and Data Confidentiality statements were provided.

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I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: 2,4-D isopropyl ester
   Description: combination amber liquid and off-white solid
   Lot/Batch #: *918009*
   Purity: 99.4% a.i.[bulk material: 97.12% pure; correction factor: 1.23 (adjust for purity & acid equivalents)]
   CAS #: 94-11-1

2. Vehicle: Mazola® 100% corn oil
   Description: yellow viscous liquid
   Lot/Batch #: Not provided

3. Test animals: Species: Rat
   Strain: Sprague-Dawley Crl:CD®BR
   Age at mating: ≈12 weeks old (86 days old)
   Weight at mating: 209-274 grams on gestation day 0; [216-275 grams (range-finding)]
   Source: Charles River Breeding Laboratories, Inc., Portage, Michigan
   Housing: Individually in suspended wire mesh cages.
   Diet: Purina® Certified Rodent Chow® #5002, ad libitum
   Water: municipal water, ad libitum
   Environmental conditions:
   Temperature: 72±4° F
   Humidity: 30-70%
   Air changes: 10/hr
   Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period (P):11 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates - start: 4/12/94 end: 5/6/94; range-finding: start: 3/1/94 end: 3/24/94

2. Mating: Females were placed in a suspended wiremesh cage with a resident male [untreated, sexually mature] from the same strain and source for breeding. Positive evidence of mating was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. The day on which evidence of mating was identified was termed day 0 of gestation , and the animals were separated.

3. Animal Assignment: The randomization procedure by which the bred rats were consecutively assigned in a block design to groups containing 25 rats each was as follows: the first mated

female and the appropriate gestation day 0 designation were recorded and the female was assigned to group 1, the second mated female was assigned to group 2 and the third to group 3, etc. This process was continued daily until 25 females were placed into each group (Table 1).

TABLE 1. Animal Assignment

Dose (mg/kg bw/day)♪	0	10	30	100
# Females	25	25	25	25

♪ acid equivalents

4. Dose selection rationale: The dose levels selected for the definitive developmental toxicity study (Table 1) were based on the results of the range-finding study [MRID 43523001] in which 2,4-D isopropyl ester was administered orally via gavage to 8 pregnant female rats/dose at dosages of 0, 25, 50, 100, 200, 400 mg/kg/day on gestational days [GD] 6 through 15. There was no adverse effect on pregnancy rate at any dose level. There was severe maternal toxicity at the 200 [mortality on GD 9 and 11 (2 dams), gait alterations (rocking, lurching, or swaying)] and 400 [mortality between GD 7 and 11 (all dams); body-weight loss (-9 grams between GD 6-7), decreased food consumption, gait alterations (rocking, lurching, or swaying), impaired use of hindlimbs, prostration; reddened adrenals and cortico-medullary junctions in kidneys] mg/kg/day dose levels. Postimplantation loss was severely increased at 200 mg/kg/day, and mean fetal body weight was reduced. One 200 mg/kg/day fetus had an omphalocele, and two fetuses in one litter had localized fetal edema of the neck and thorax. Based of the results of this study, dose levels of 10, 30, and 100 mg/kg/day were selected for the definitive study.

5. Dosage preparation and analysis

Prior to preparation, the test material was placed in an incubator [≈50°C for 2 hours]. The appropriate amount of test material was weighed for each group, and a sufficient amount of vehicle [Mazola® corn oil] was added. The preparations were stirred [magnetic stir plate] throughout the sampling and dosing procedures. Preparations for all dose groups were made twice [4/15/94 and 4/24/94] and were stored at room temperature. Prior to the start of the study, three sets of duplicate samples were collected from the top, middle, and bottom of a representative series of solutions for the four groups. One set of samples was analyzed for homogeneity, and the remaining sets were combined and analyzed for 8-day stability. On dose -preparations days, samples were collected from the middle stratum of each dosing formulation, and these were analyzed for concentration.

Results - Homogeneity Analysis: All groups met with WIL SOP requirements for homogeneity; i.e., the group mean differed by less than 15% from the target dose

concentration for that group and the location means for each group differed by less than 10% from the overall mean for that group.

Stability Analysis: After eight days of storage, there was no apparent degradation. The mean concentrations were 101% to 102% of the target concentrations.

Concentration Analysis: All dose formulations were 95.3%-104% of the target concentration.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered once daily by gavage, on gestation days 6 through 15, in a volume of 5 mL/kg of body weight. Dosing was based on the most recent body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for moribundity and mortality twice daily and daily for clinical signs [GD 0-20]. On the first dosing day, the animals were observed for signs of toxicity ≈ 1, 2, and 4 hours post dose. Since no remarkable clinical signs were observed at 2 and 4 hours post dose, only the 1-hour post dose observation period continued throughout dosing. Body weight data were recorded on gestation days 0, 6 through 16, and 20, and body-weight changes were calculated for various intervals. Gravid uterine weight was determined and net body weight and net body-weight change were calculated. Food consumption data were recorded on gestation days 0, 6-16, and 20. All surviving females were euthanized by carbon dioxide inhalation on gestation day 20. The thoracic, abdominal, and pelvis cavities were opened and the contents examined. The liver was excised and weighed, and sections from each liver were preserved for possible future histopathological examination. The uterus and ovaries were excised, and the number of corpora lutea on each ovary was recorded. The trimmed uterus was weighed, opened, and the number and location of all fetuses, early and late resorptions, and the total number of implantation sites were recorded. Uteri with no macroscopic evidence of nidation were excised, opened, and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss. Intrauterine data were summarized using two methods of calculation:

Group Mean Litter Basis:

Post-implantation Loss/Litter = $\frac{\text{\# dead fetuses, resorptions (early/late)/group}}{\text{\# gravid females/group}}$

Proportional Litter Basis:

$$\textbf{Summation per Group} = \frac{\text{Post-implantation loss/litter (\%)}♪}{\text{\# of litters/group}}$$

$$♪ = \frac{\text{\# dead fetuses, resorptions (early/late)/litter}}{\text{\# implantation sites/litter}} \times 100$$

2. Fetal Evaluations - The fetuses were examined in the following manner: Each fetus was sexed, weighed, and tagged [WIL study #, dam #, and fetus #]. A detailed external examination of each fetus was conducted to include the eyes, palate and external orifices. Crown-rump measurements were recorded for late resorptions. Each fetus was examined viscerally by a modification of the Stuckhardt and Poppe√ fresh dissection technique to include the heart and major vessels. The sex of each fetus was verified by an internal examination. Fetal kidneys were examined and graded for renal papillae development by a method by Woo and Hoar. Heads from approximately one-half of the fetuses in each litter were placed in Bouin's fixative for subsequent soft-tissue examination by the Wilson sectioning technique. The heads from the remaining fetuses were examined by a mid-coronal slice. All carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described in Dawson. The skeletal examination was conducted utilizing low power magnification provided by a stereo microscope. External, visceral, and skeletal findings were recorded as developmental variations or malformations. Fetal developmental findings were summarized by (1) presenting the incidence of a given finding both as a percentage of the number of fetuses and the number of litters available for examination in the group; and (2) considering the litter as the basic unit for comparison and calculating the number of affected fetuses in a litter on a proportional basis as follows:

$$\text{Summation per group (\%)} = \frac{\text{Viable fetuses affected/litter (\%)}√}{\text{\# of litters/group}}$$

$$√ = \frac{\text{\# viable fetuses affected/litter}}{\text{\# viable fetuses/litter}} \times 100$$

D. DATA ANALYSIS

1. Statistical analyses: All data collected were subjected to routine appropriate statistical procedures. The statistical tests were performed by a Digital® MicroVAX® 3400 computer with appropriate programming.

2. Indices: None provided.

3. Historical control data: Historical control data were submitted [APPENDIX B (pages 212-

219) and APPENDIX C (pages 220-298).

II. RESULTS

A. MATERNAL TOXICITY

1. **Mortality and Clinical Observations**: One high-dose dam died on gestation day 17, and clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis] were observed in the dam at the daily and/or one-hour post dose observation one to five days prior to death. Two additional high-dose dams displayed similar clinical signs [rocking, lurching, or swaying] between gestation days 11 and 14. There were no treatment-related effects noted in mortality or clinical signs in the other treated dams. Deaths and similar clinical signs were observed in the range-finding study at 200 mg/kg/day and 400 mg/kg/day but not at 25, 50, or 100 mg/kg/day.

2. **Body Weight** - At the high-dose level, a statistically-significant [slight (93%-96% of control)] decrease was observed in body weight from gestation day [GD] 7 through GD 20. At termination, body weight of the high-dose dams was 95% of the control value. All groups initially lost weight [GD 6-7], and the losses were statistically-significantly greater than the control [-1 gram] at all dose levels [-6, -6, and -9 grams with increasing dose]. Thereafter, only the high-dose group displayed a significant reduction in body-weight gain [50%-85% of control] compared to the control [Table 2]. There was a dose-related decrease in body-weight gain during the dosing period [days 6-15], with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. At the high-dose level, body-weight gains during the post-treatment phase [days 16-20] were only slightly lower [95% of control] than the control. Corrected body-weight gains at the high-dose level were significantly lower [83% of control] than control.

TABLE 2. Mean (±SD) Maternal Body Weight Gain (g) [a]

Interval	Dose in mg/kg bw/day (# of Dams)			
	Control 0 (25)	10 (24)	30 (25)	100 (22)
Pretreatment:	(25)	(24)	(25)	(22)
Days 0-6	36.0±8.5	33.0±5.0	34.0±5.9	33.0±5.6
Treatment:	(25)	(24)	(25)	(22)
Days 6-7	-1±4.2	-6**±5.9	-6**±5.0	-9**±7.6
Days 7-8	4±5.4	6±5.8	4±4.7	3±6.3 [75]
Days 8-9	2±5.0	3±4.4	4±5.2	3±6.4
Days 9-10	6±4.1	6±6.6	4±4.4	3±7.0 [50]
Days 12-13	5±4.8	5±5.0	5±4.6	3±5.7 [60]
Days 13-14	5±4.0	5±3.9	4±5.9	4±5.2 [80]
Days 6-9	6±6.4	4±7.6 [67]♪	2±4.6 [33]	-3**±10.8
Days 6-16	53±8.0	49±8.7	43*±7.2 [81]	33**±22.8 [62]
Days 0-20	147±18.1	143±15.0	135±14.6 [92]	125**±26.6 [85]
Posttreatment:	(25)	(24)	(25)	(21)
Days 16-20	58±11.1	61±9.2	58±8.4	55±18.0 [95]
Corrected BW Gain	70.7±13.2	66.5±9.0	64.9±11.8 [92]	58.9**±9.8 [83]

a Data obtained from Tables 6-7, pages 47-49 in the study report; * Statistically different ($p < 0.05$) from the control.
** Statistically different ($p < 0.01$) from the control; ♪ [% of control]

3. <u>Food Consumption</u> - There was a dose-related, statistically-significant, decrease [Table 3] in food consumption initially [GD 6 to 7; all dose levels on a g/rat/day basis] and throughout the dosing period [GD 6-16; mid- and high-dose levels].

Table 3. Mean maternal food consumption (g/rat/day ± SD).[a]

Interval	Dose in mg/kg/day			
	0	10	30	100
Pretreatment:				
Days 0-6	22±2.0	22±1.6	22±1.9	21±1.8
Treatment:				
Days 6-7	20±2.1	17*±3.6 [85]	16**±4.1 [80]	14**±4.0 [70]
Days 7-8	19±4.0	19±4.2	18±3.4	15**±3.5 [79]
Days 8-9	21±3.9	20±4.3	19±2.6	16±**±5.2 [76]
Days 9-10	21±2.6	22±3.0	20±2.2	17**±5.1 [81]
Days 12-13	23±3.6	24±3.3	21±1.8	19**±4.8 [83]
Days 13-14	22±2.4	22±2.2	20±4.2	18**±5.4 [82]
Days 6-9	20±2.6	19±3.5	18±2.6	15**±3.4 [75]
Days 6-16	22±2.0	21±1.9	20*±1.4 [91]	17**±3.6 [77]
Days 0-20	23±1.8	23±1.6	22*±1.5	21**±1.7 [91]
Posttreatment				
Days 16-20	29±2.2	29±1.8	29±2.2	28±2.4

a Data extracted from study report, Tables 8 and 9, pages 50-53; * $p<0.05$; ** $p<0.01$

4. **Gross Pathology** - There were no treatment-related gross pathologic findings at necropsy.

5. **Cesarean Section Data** - The mean numbers of corpora lutea, implantation sites, and viable fetuses were comparable among the groups [Table 4]. There were no dead fetuses. The mean number of early resorptions was comparable among the groups, and only the mid- [2] and high- [1] dose groups had late resorptions. Preimplantation loss was comparable among the groups, and there was only a slight increase in postimplantation loss at the mid- [1.3] and high-[1.5] dose levels compared to the control [1.0] and low-dose [0.9] groups. One high-dose dam [4.6%] had 100% resorptions compared to none in the control or other dose groups. The historical control incidence of total litter resorptions is 8 out of 2676 litters [0.3%]. There was a significant decrease in the number of female fetuses at the high-dose level [5.3] compared to the control [6.8], and the % females at the high-dose level [40%] was below the historical control range [43.3%-58.4%]. Fetal body weight was significantly lower [89% of control] at the high-dose level compared to the control.

TABLE 4 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	10	30	100
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	25	24	25	22
Pregnancy Rate (%)	(100)	(96)	(100)	(88)
# Nonpregnant	0	1	0	3
Maternal Wastage				
# Died	0	0	0	1
# Died Pregnant	0	0	0	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea	402	394	407	345
Corpora Lutea/Dam	16.8±2.6	16.4±1.8	16.3±2.6	16.43.9
Total # Implantations	364	351	351	303
(Implantations/Dam)	14.6±3.2	14.6±1.8	14.0±2.1	14.4±3.4
Total # Litters	25	24	25	21
Total # Live Fetuses	339	329	319	272
(Live Fetuses/Dam)	13.6±3.6	13.7±2.5	12.8±2.5	13.0±5.0
Viable Fetuses (%)	91.7±11.3	93.2±8.8	90.7±11.3	89.0±26.9
Total # Dead Fetuses	0	0	0	0
(Dead Fetuses/Dam)	0	0	0	0
Total # Resorptions	25	22	32	31
Early	25	22	30	30
Late	0	0	2	1
Resorptions/Dam				
Early [%]	1.0±1.0 [8±11]	0.9±1.1 [7±9]	1.2±1.3 [9±9]	1.4±3.3 [11±25]
Late [%]	0	0	0.1±0.4 [1±4]	0.0±0.2 [0.4±2]
Litters with Total Resorptions	0	0	0	1 [4.6%]

Observation	Dose (mg/kg bw/day)			
	0	10	30	100
Mean Fetal Weight (g)	3.6±0.2	3.6±0.2	3.4±0.3	3.2**±0.3
Males	3.7±0.2	3.7±0.2	3.5±0.3	3.3**±0.3
Females	3.5±0.2	3.4±0.2	3.4±0.3	3.1**±0.2
Sex Ratio (% Male) # males/# females	(51) 168/171	(56) 184/145	(52) 164/155	(60) 161/111
Mean # males/# females	6.7/6.8	7.7/6.0	6.6/6.2	7.7/5.3*
Preimplantation Loss (%)	52 [2.3±2.7]	43 [1.8±1.3]	56 [2.2±2.6]	42 [2.0±1.9]
Postimplantation Loss (%)	25 [1.0±1.0]	22 [0.9±1.1]	32 [1.3±1.5]	31 [1.5±3.4]
mean (%)	8.3±11.3	6.8±8.8	9.3±11.3	11.0±26.9

a Data obtained from Tables 11-12, pages 55-58, Table 29, pages 133-136, and Table 30, pages 137-140] in the study report.
* $p < 0.05$); ** $p < 0.01$.

B. DEVELOPMENTAL TOXICITY

1. External Examination - There were no treatment-related external malformations or variations.

2. Visceral Examination -There were no treatment-related visceral malformations or variations.

3. Skeletal Examination -According to the study report, the only skeletal malformation observed at the high-dose level was one fetus/one litter with vertebral anomaly with or without associated rib anomaly. However, the presence of 7th cervical rib(s) and 14th rudimentary rib(s) are considered skeletal malformations also, and there was an increased [statistically-significant] incidence of both at the high-dose level compared to the control incidence [Table 5]. Skeletal variations [hyoid unossified, 25 presacral vertebrae] were slightly increased at the high-dose level compared to the control, and bent ribs were increased at the mid- and high-dose levels compared to the control, although there was no dose-response. The incidence of 7th cervical rib(s) is outside the historical control data [Table 6] on both a fetal and litter basis.

Table 5. Malformations and Variations

Dose/Finding	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
# fetuses/# litters examined	339/25	329/24	319/25	272/20
skeletal malformation	# fetuses/# litters	# fetuses/# litters	# fetuses/#	# fetuses/# litters
filamentous tail	1/1	0	litters	0
vertebral anomaly with or without associated rib anomaly	0	1/1	0	1/1
bent limb bone	0	1/1	0	0
only 12 full pairs of ribs	0	1/1	0	0
total skeletal malformations [reported]	1/1	2/2	0	1/1
presence of 7th cervical ribs	3 [0.9]✖/3 [12]	9 [2.7]/6 [25]	1/1	15 [5.5]/8* [40]
presence of 14th rudimentary ribs	3 [0.9]/2 [8]	4 [1.2]/3 [13]	5 [1.6]/3 [12] 6 [1.9]/5 [20]	26 [9.6]/13* [65]

Table 5. Malformations and Variations				
Dose/Finding	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
# fetuses/# litters examined	339/25	329/24	319/25	272/20
skeletal variations *hyoid unossified* *25 presacral vertebrae* *bent ribs*	# fetuses/# litters 4 [1.2]/2 [8.0] 0/0 2 [0.6]/2 [8.0]	# fetuses/# litters 4 [1.2]/3 [12.5] 0/0 3 [0.9]/2 [8.3]	# fetuses/# litters 0/0 0/0 9 [2.8]/8 [32]	# fetuses/# litters 4 [1.5]/3 [15] 1 [0.4]/1 [5.0] 5 [1.8]/5 [25]

✖[%]; data from Tables 13-17, pages 59-66 of the report

Table 6. Historical Control Data		
	# fetuses [24950]	# litters [2403]
skeletal malformation vertebral anomaly with or without associated rib anomaly presence of 7th cervical ribs presence of 14th rudimentary ribs	 12 (0-0.8)✖ 116 (0-3.3) 1349 (0-38.9)	 12 (0-5.0) 94 (0-27.3) 634 (0-94.7)
skeletal variations *hyoid unossified* *25 presacral vertebrae* *bent ribs*	 175 (0-38.9) 45 (0-4.0) 178 (0-4.2)	 117 (0-50) 32 (0-22.7) 120 (0-30)

✖ (%) data from pages 216 and 219 of the report

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS: Maternal toxicity was exhibited at the 100 mg/kg/day dose level, where one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas, red material on the urogenital area, and ptosis], and decreased body-weight gains and food consumption were observed throughout the dosing period. Decreased body-weight gains and food consumption were also observed at the mid-dose level [30 mg/kg/day].

Developmental toxicity was observed at 30 and 100 mg/kg/day, where increased postimplantation losses and/or decreased mean fetal body weights were observed. At the high-dose level, an increased incidence of two fetal skeletal developmental variations [7th cervical ribs and 14th rudimentary ribs] was considered a possible effect on fetal development. The only skeletal malformation reported at the high-dose level was one fetus/one litter with vertebral anomaly with or without associated rib anomaly. The NOAEL for both maternal and developmental toxicity was considered to be 10 mg/kg/day.

B. REVIEWER'S DISCUSSION: 1.MATERNAL TOXICITY: There were no abortions or premature deliveries. There was one death; a high-dose dam died on gestation day 17. Clinical signs observed in this dam included rocking, lurching, or swaying, decreased defecation, clear matting on the ventral abdominal and thoracic areas and ptosis. Treatment-

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related clinical signs were also observed in two other high-dose dams and included rocking, lurching, or swaying, which were observed at the one-hour post-dose examination period between gestation days 11 and 14. At the high-dose level, a slight, statistically-significant [93%-96% of control], decrease was observed in body weight from gestation day [GD] 7 through GD 20. At termination, body weight of the high-dose dams was 95% of the control value. All groups initially lost weight [GD 6-7]. Thereafter, only the high-dose group displayed a significant reduction in body weight [50%-75% of control] compared to the control. There was a dose-related decrease in body-weight gain during the dosing period, with the mid- [81% of control] and high- [62% of control] dose deficits attaining statistical significance. Corrected body-weight gains at the high-dose level were significantly lower [83% of control] than control. Decreased food consumption was noted at all dose levels initially [GD 6-7], but thereafter only the high-dose displayed decreased food consumption [70%-83% of control] during the dosing period.

The mean numbers of corpora lutea, implantation sites, and viable fetuses were comparable among the groups. There were no dead fetuses. The mean number of early resorptions was comparable among the groups, and only the mid- [2] and high- [1] dose groups had late resorptions. Preimplantation loss was comparable among the groups, and there was only a slight increase in postimplantation loss at the mid- [1.3] and high-[1.5] dose levels compared to the control [1.0] and low-dose [0.9] groups. One high-dose dam [4.6%] had 100% resorptions [10 early, 1 late] compared to none in the control or other dose groups. The historical control incidence of total litter resorptions is 8 out of 2676 litters [0.3%].

2. DEVELOPMENTAL TOXICITY: Fetal body weight was significantly lower [89% of control] at the high-dose level compared to the control. There was a significant decrease in the number of female fetuses at the high-dose level [5.3] compared to the control [6.8], and the % females at the high-dose level [40%] was below the historical control range [43.3%-58.4%].

There were no treatment-related external or visceral malformations or variations. The presence of 7th cervical rib(s) and 14th rudimentary rib(s) are considered skeletal malformations, and there was an increased [statistically-significant] incidence of both at the high-dose level compared to the control incidence. The incidence of 7th cervical rib(s) is outside the historical control data on both a fetal and litter basis. Skeletal variations [hyoid unossified, 25 presacral vertebrae] were slightly increased at the high-dose level compared to the control, and bent ribs were increased at the mid- and high-dose levels compared to the control, although there was no dose-response. Similar skeletal findings were observed in the rat developmental toxicity studies on the parent compound and amine salts and other esters of 2,4-D.

The maternal toxicity NOAEL is 10 mg/kg/day, and the maternal toxicity LOAEL is 30 mg/kg/day, based on decreased body-weight gain and food consumption during the dosing period. At the highest dose tested [100 mg/kg/day], one death, clinical signs [rocking, lurching, or swaying, decreased defecation, clear matting on the ventral

abdominal and thoracic areas, red material on the urogenital area, and ptosis], and one litter with 100% resorptions were observed.

The NOAEL for developmental toxicity is 30 mg/kg/day, and the developmental toxicity LOAEL is 100 mg/kg/day, based on a decreased fetal body weight, an increased incidence of skeletal malformations, and a decreased number of female fetuses.

This guideline developmental toxicity study is classified Acceptable/Guideline, and it satisfies the guideline [OPPTS 870.3700; §83-3(a)] requirement for a developmental toxicity study in the rodent.

C. STUDY DEFICIENCIES: None that would adversely affect study interpretation.